03-13-00

A)

UTILITY PATENT APPLICATION TRANSMITTAL (only for continuation and divisional applications under 37 CFR 1.53(b))	Docket No.: P-IM 4082 **Prior Application Info:** Examiner: Y. Ryan Group/Art Unit: 1641

Address to: ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
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CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Steven Hsieh
(TYPED OR PRINTED NAME OR PERSON MAILING PAPER OR FEE)

Steven Hsieh
(SIGNATURE OF PERSON MAILING PAPER OR FEE)

This is a request for filing a
X continuation ___ divisional

application under 37 CFR 1.53(b), of pending prior application serial no. 08/482,454, filed June 6, 1995,(list only immediate prior application).

Title: METHOD FOR INCREASING HDL CHOLESTEROL LEVEL
Inventor(s)(full name of each inventor): Deborah Y. Kwoh, Steven W. Brostoff and Dennis J. Carlo

No abandonment of, or termination of proceedings, has occurred in the above-identified prior application.

1. X An application based on the prior application as filed and containing no new matter is enclosed, consisting of:
   1 page application cover sheet
   9 pages of specification (includes claims and abstract)
   0 sheets of drawing(s).

2. X 3 pages of a copy of the oath or declaration filed on October 9, 1995, from prior application (37 CFR 1.63(d)), U.S. serial no. 08/482,454, filed June 6, 1995, is enclosed.

3. ___ A signed statement DELETING inventor(s) named in the prior application (37 CFR 1.63(d)(2) and 1.33(b)) is attached.

4. ___ Nucleotide and/or Amino Acid Sequence Submission is enclosed:
   ____ computer readable copy of sequence listing
   ____ paper copy of sequence listing, pages ____ through ___
   ____ Statement Under _____
   ____ Request to Use Computer Readable Form of Sequence Listing From Another Application
   ____ Other:

5. _X_ Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under item no. 2 of this form, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference herein.

6. ___ Enter the unentered amendment previously filed on _____ under 37 CFR 1.116 in the prior application.

7. ___ A preliminary amendment is enclosed.

8. Small entity status:
   a. ___ A small entity statement is enclosed.
   b. _X_ A small entity statement was filed in prior application serial no. 08/482,454, and such status is still proper and desired.
   c. ___ is no longer claimed.

9. _X_ Amend the specification by:
   _X_ inserting before the first paragraph on page 1: or
   ___ deleting the paragraph on page 1 regarding related applications and inserting therefor:

   This application is a continuation, of application serial no. 08/482,454, filed June 6, 1995.

10. ___ Cancel in this application original claims _____ _____of the prior application before calculating the filing fee. (At least one original independent claims must be retained for filing purposes.)

20. X The Commissioner is hereby authorized to charge fees under 37 CFR 1.16 and 1.17 which may be required or credit any overpayment to Deposit Account No. 03-0370. A duplicate copy of this sheet is enclosed.

Address all future communications to:

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Respectfully submitted,

Date: March 10, 2000

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___ assignee of complete interest

X attorney or agent of record

___ filed under Sec. 1.34(a)

Initial Information Data Sheet

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Application Information

Title Line One :: **METHOD FOR INCREASING HDL CHOLESTEROL**
Title Line Two :: **LEVEL**
Total Drawing Sheets :: **None**
Application Type :: **Utility**
Docket Number :: **P-IM 4082**

Representative Information

Registration Number One :: **31,815**
Registration Number Two :: **34,949**
Registration Number Three :: **30,806**
Registration Number Four :: **38,701**
Registration Number Five :: **36,933**
Registration Number Six :: **39,200**
Registration Number Seven :: **38,444**
Registration Number Eight :: **37,915**
Registration Number Nine :: **41,029**
Registration Number Ten :: **44,048**
Registration Number Eleven:: **43,947**
Registration Number Twelve:: **45,201**

Continuity Information

This application is a :: **Continuation, of**
> Application One :: **08/482,454**
Filing Date :: **June 6, 1995**

Document: INITIAL INFORMATION DATA SHEET
Attorney Docket No: P-IM 4802

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Steven Hsieh
Printed Name of Person Mailing Paper or Fee

Steven Hsieh
Signature of Person Mailing Paper or Fee

APPLICATION

for

UNITED STATES LETTERS PATENT

on

METHOD FOR INCREASING HDL CHOLESTEROL LEVEL

by

Deborah Y. Kwoh

Steven W. Brostoff

and

Dennis J. Carlo

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Steven Hsieh

(TYPED OR PRINTED NAME OR PERSON MAILING PAPER OR FEE)

(SIGNATURE OF PERSON MAILING PAPER OR FEE)

Sheets of Drawings: None

Docket No.: P-IM 4082

Attorneys

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METHOD FOR INCREASING HDL CHOLESTEROL LEVEL

This invention relates generally to the field of immunotherapy and, more specifically, to methods of stimulating an immune response to cholesteryl ester transfer protein (CETP).

BACKGROUND OF THE INVENTION

Blood cholesterol levels have long been thought to correlate directly with risk of atherosclerotic cardiac disease, the leading cause of heart attacks. More recently, it has been appreciated that blood cholesterol is actually composed of two primary forms: the high density lipoproteins (HDL) and low density lipoproteins (LDL). Rather than being associated with the disease risk, high HDL levels are apparently inversely predictive. In fact, studies have now indicated that HDL has a direct action in protecting against atherosclerosis and may even promote atherosclerosis plaque regression.

Numerous factors are involved in regulating the level of cholesterol in the body. Cholesteryl ester transfer protein (CETP) is an enzyme responsible for transporting cholesterol esters (CE) from HDL to very low density lipoproteins (VLDL) and LDL. VLDL's are eventually converted into LDL. CETP accelerates specifically the exchange of lipid components between pro- and anti-atherogenic lipo protein tractions. In particular, there is a strong inverse correlation between the levels of CETP in the plasma and the levels of HDL cholesterol. CETP activity levels are elevated in individuals suffering from dietary or genetic hypercholesterolemia. Increased levels of CETP activity result in lowered levels of HDL. In contrast, individuals with deficiencies in CETP activity due to mutations in the CETP gene have markedly elevated HDL levels.

The immune systems of higher organisms developed as a means for protecting the individual against invasion by deleterious foreign materials such as viruses, bacteria and parasites. Cells of the immune system are able to distinguish between materials from the individuals own body (termed "self" materials) and foreign material, or antigens. When foreign material enters the body, the immune system mounts a response. Antibodies that specifically recognize and bind to the foreign material are produced (the antibody or humoral response.) In addition, T cells are mobilized to repel the foreign substance (the T cell or cellular response.) Materials which are recognized as self do not normally stimulate such responses except in certain pathological conditions, primarily auto-immune disease. Even where the presence of an endogenous protein is itself deleterious, the immune system cannot serve as a regulator if the material is recognized as self.

Because of HDL'S potentially beneficial effect in preventing atherosclerosis, there exists a need for methods which can be used to increase its level in the serum. Such methods should ideally be specific and reliable and involve as little invasion of the body as possible. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method for increasing HDL cholesterol in a mammal by stimulating an immune response that inhibits the function of CETP. Such an immune response can be induced by immunizing with CETP or fragments of CETP (together termed "CETP Peptides") which contain an epitope capable of stimulating such a response. The peptides can be conjugated to a carrier, such as Keyhole Limpet Hemocyanin (KLH) or ovalbumin, in

order to increase immunogenicity. Adjuvants can also be administered.

In one embodiment, the fragments of CETP used to raise the antibody response are about ten to twenty amino acids in length and contain sequences homologous to the sequence in rabbit or human CETP.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a means to utilize the body's own immune system to lower CETP levels, thereby increasing the level of beneficial HDL cholesterol. The invention provides an effective method of raising HDL in the blood or more specifically, the serum. By utilizing the body's own immune system to increase HDL levels, the invention avoids the problems associated with the repeated administration of drugs, which have undesirable side effects.

According to the present invention, CETP peptide is administered to an appropriate individual in such a manner as to elicit an anti-CETP immune response. The CETP can be chosen to contain an epitope capable of stimulating an antibody or humoral response. Alternatively, the CETP can stimulate a cellular response, or other immune response. CETP peptides can be elected to contain B cell epitopes, sequences capable of stimulating the production of antibodies that specifically recognize and bind to the epitope. Alternatively, CETP peptides can be chosen which stimulate a T cell or more general immune response.

Individuals exhibiting, or at risk of exhibiting, low serum levels of HDL cholesterol are particularly appropriate for such treatment. Serum HDL levels can be determined using methods well-known in the

art (See Warnick, G.R. J.Lipid. Res., 19:65 (1978), for example, which is incorporated herein by reference). Serum HDL of less than about 30-35 mg/dl is considered low. Subjects exhibiting a serum HDL level below this level are particularly suitable for the treatment of the invention.

The protein or peptide to be administered can be all or part of the CETP protein, so long as the protein or peptide contains a B cell and/or T cell epitope. As used herein, "CETP peptide" is intended to include both the full length CETP amino acid sequence as well as fragments thereof. The peptides can have a sequence corresponding to or homologous to a mammalian CETP sequence. It will be appreciated that the peptide can differ from the native sequence to some extent so long as it is capable of inducing antibodies that inhibit the activity of CETP.

CETP is a 55 kD protein based on its amino acid sequence, but with post-translational modifications it has an apparent molecular weight of 66-74 kD. The human CETP mRNA sequence is available in Genbank (accession number M30185). The rabbit CETP mRNA sequence is available in Genbank (accession number M27486). The genbank sequences were translated using the MacVector software program (I.B.M., New Haven, Connecticut) to obtain the complete amino acids sequence of human and rabbit CETP.

Because CETP and its peptide derivatives may be recognized are "self" antigens, carriers can be used to increase their immunogenicity. Such carriers are well known in the art and include, for example, such compounds as Keyhole Limpet Hemocyanin (KLH), ovalbumin and Diphtheria toxoid (Wako BioProducts). The CETP peptides can be conjugated to such carriers by methods well-known

in the art. See Current Protocols in Molecular Biology, Ausebell, Brent, Kingston, Moore, Seidman, Smith & Strull eds. (1987), or manufacturers' instructions, which is incorporated herein by reference. The immunogenicity of the peptides can be also increased by administration of a adjuvant. Various adjuvants are well-known and available. See Antibodies: A Laboratory Manual, Harlow and Lane eds., (1988) which is incorporated herein by reference.

The extent of the anti-CETP response induced by the administration of the CETP peptides can be monitored using a variety of assays. For example, competitive format immunoassays can be employed using anti-CETP antibodies or anti-CETP antiserum. Alternatively, the activity level of the CETP in the subject individual can be monitored using, for example a ^{3}H-cholesterol oleate transfer assay. Lasuncion, M.A., et al. Biochem J., 270:441-449 (1990). Reduction in CETP activity is an indirect indication of the anti-CETP response.

The following examples are intended to illustrate but not limit the invention.

Example 1

Administration of CETP peptide immunogen

Peptides corresponding to portions of the human, rabbit and rabbit/human CETP were prepared according to standard peptide synthesis protocols. The following peptide sequences were prepared:

H-Cys-Asp-Ser-Gly-Arg-Val-Arg-Thr-Asp-Ala-Pro-Asp-OH (SEQ ID No.: 1)

H-Cys-Asp-Ala-Gly-Ser-Val-Arg-Thr-Asn-Ala-Pro-Asp-OH (SEQ ID No.: 2)

H-His-Leu-Leu-Val-Asp-Phe-Leu-Gln-Ser-Leu-Ser-OH. (SEQ ID No.: 3)

The first peptide (SEQ ID 1) is taken from the Human CETP peptide sequence (residues 131-142 without signal peptide) from Smith and Barakat, Med. Sci. Res., 21:911-912 (1993), which is incorporated herein by reference. The second peptide (SEQ ID 2) is the corresponding rabbit sequence and differs by only 3 amino acids from the human.

The third peptide (SEQ ID 3) is common to both human and rabbit and is an epitope recognized by anti-CETP-monoclonal antibody which is neutralizing. Tall, A.R., J. Lipids Res., 34:1255-1257 (1993).

The peptides were conjugated to ovalbumin by the procedure of Current Protocols in Molecular Biology, supra. Of four New Zealand White rabbits, approximately four months of age, two were injected intramuscularly with 100 micrograms of the ovalbumin-conjugated human peptide (Seq. ID No.: 1) and CFA in PBS saline and two were injected with the equivalent human/rabbit peptide (Seq. ID No. 3). The animals were boosted twice at one month intervals with with the same peptides in IFA..

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made withouth departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. A method of stimulating an immune response to increase HDL cholesterol in a mammal exhibiting low levels of serum HDL comprising administering to said mammal a composition comprising an immunogenic epitope of CETP.

2. The method of claim 1, wherein said composition is substantially purified CETP.

3. The method of claim 1, wherein said composition is a peptide.

4. The method of claim 1, wherein said composition contains a B cell epitope.

5. The method of claim 3, wherein said peptide is:

H-Cys-Asp-Ala-Gly-Ser-Val-Arg-Thr-Asn-Ala-Pro-Asp-OH

H-Cys-Asp-Ser-Gly-Arg-Val-Arg-Thr-Asp-Ala-Pro-Asp-OH

H-His-Leu-Leu-Val-Asp-Phe-Leu-Gln-Ser-Leu-Ser-OH.

6. The method of claim 1, wherein said composition comprises a carrier.

7. The method of claim 5, wherein said carrier is selected from the group consisting of KLH, ovalbumin and Diphtheria toxoid.

8. The method of claim 1, wherein said composition is administered with an adjuvant.

9. The method of claim 1, wherein said administration is repeated.

ABSTRACT OF THE DISLOSURE

The present invention provides a method for increasing HDL cholesterol in a mammal by stimulating an immune response that inhibits the function of CETP. Such an immune response can be induced by immunizing with CETP or fragments of CETP (together termed "CETP Peptides") which contain an epitope capable of stimulating such a response. The peptides can be conjugated to a carrier, such as KLH or ovalbumin, in order to increase immunogenicity. Adjuvants can also be administered.

COPY

DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

As the below-named inventors, we hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD FOR INCREASING HDL CHOLESTROL LEVEL, the specification of which

_____ is attached hereto.
__X__ was filed on June 6, 1995, (Attorney Docket No. P-IM 1618)
as Application Serial No. 08/482,454
and was amended on (or amended through) _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

willful false statements may jeopardize the validity of the application or any patent issued thereon.

We hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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